Claims

1. A method for producing an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a yeast cell medium comprising the IGF polypeptide, wherein the method comprises:

(a) performing a first cation exchange chromatography with the yeast cell medium to yield a first IGF mixture;

(b) denaturing and renaturing IGF species present in the first IGF mixture to yield a second IGF mixture;

(c) subjecting the second IGF mixture to hydrophobic interaction chromatography to yield a third IGF mixture; and

(d) performing reverse phase high performance liquid chromatography on the third IGF mixture to yield a fourth IGF mixture, wherein the fourth IGF mixture has a greater amount of authentic, properly folded IGF than the first IGF mixture.

2. The method of claim 1, wherein the method further comprises performing a second cation exchange chromatography with the third IGF mixture prior to performing reverse phase high performance liquid chromatography.

3. The method of claim 1, wherein the method further comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 8 to about pH 12, prior to the first cation exchange chromatography.

4. The method of claim 3, wherein the method comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 10 to about pH 11, prior to the first cation exchange chromatography.

5. The method of claim 1, wherein the first cation exchange chromatography is performed using a sulfopropylated matrix.

6. The method of claim 2, wherein the second cation exchange chromatography is performed using a sulfopropylated matrix.

7. The method of claim 1, wherein the denaturing and renaturing steps are performed together using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds.

8. The method of claim 7, wherein the denaturation buffer comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

9. The method of claim 8, wherein the denaturation buffer comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

10. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

11. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

12. The method of claim 1, wherein the reverse phase high performance liquid chromatography is performed using a C_8 silica-derivatized resin.

13. The method of claim 1, wherein the yeast cell is *Pichia sp.*

14. The method of claim 13, wherein the yeast cell is *P. pastoris*.

15. The method of claim 1, wherein the yeast cell is *Saccharomyces sp.*

16. The method of claim 15, wherein the yeast cell is *S. cerevisiae*.

17. The method of claim 1, wherein the IGF is IGF-I.

18. The method of claim 1, wherein the IGF is IGF-II.

19. A method for refolding an insulin-like growth factor (IGF) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF polypeptide, wherein the method comprises denaturing and renaturing IGF species present in an IGF mixture using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF polypeptide.

20. The method of claim 19, wherein the denaturing and renaturing are done together using a denaturation

buffer which comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

21. The method of claim 20, wherein the denaturation buffer has a pH of about 9 to about 11 and comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

22. The method of claim 19, wherein the denaturing and renaturing are done together and conducted for about 8 to about 24 hours at room temperature.

23. The method of claim 22, wherein the denaturing and renaturing are conducted for about 15 to about 18 hours at room temperature.

24. A method for refolding an insulin-like growth factor (IGF) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF polypeptide, wherein the method comprises denaturing and renaturing IGF species present in an IGF mixture, wherein said denaturing and renaturing are done together using a denaturation buffer having a pH of about 9 to about 11 and which comprises about 2 M urea, about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol, wherein the denaturing and renaturing are conducted for about 15 to

about 18 hours, at room temperature, thereby producing an authentic, properly folded IGF polypeptide.

add A1 add B1